

CONTROLLED EVOLUTION OF AN RNA ENZYME

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It is generally thought that prior to the origin of protein synthesis, life on earth was based on self-replicating RNA molecules. This idea has become especially popular recently due to the discovery of catalytic RNA (ribozymes). RNA has both genotypic and phenotypic properties, suggesting that it is capable of undergoing Darwinian evolution. RNA evolution is likely to have played a critical role in the early history of life on earth, and thus is important in considering the possibility of life elsewhere in the solar system. We have constructed an RNA-based evolving system in the laboratory, combining amplification and mutation of an RNA genotype with selection of a corresponding RNA phenotype. This system serves as a functional model of a primitive organism. It can also be used as a tool to explore the catalytic potential of RNA. By altering the selection constraints, we are attempting to modify the substrate specificity of an existing ribozyme in order to develop ribozymes with novel catalytic function. In this way, we hope to gain a better understanding of RNA's catalytic versatility and to assess its suitability for the role of primordial catalyst.

All of the RNA enzymes that are known to exist in contemporary biology carry out cleavage/ligation reactions involving RNA substrates. The *Tetrahymena* ribozyme, for example, catalyzes phosphoester transfer between a guanosine-containing and an oligopyrimidine-containing substrate. We tested the ability of mutant forms of the *Tetrahymena* ribozyme to carry out a comparable reaction using a DNA, rather than RNA substrate. An ensemble of structural variants of the ribozyme was prepared and tested for their ability to specifically cleave d(GGCCCTCT•A₃TA₃TA) at the phosphodiester bond following the sequence CCCTCT. We recovered a mutant form of the enzyme that cleaves DNA more efficiently than does the wild-type. Beginning with this selected mutant we have now scattered random mutations throughout the ribozyme and have begun an evolutionary search to further expand the catalytic repertoire of RNA.